Bacterial Genomic DNA Isolation Kit

rev 07/20

(Catalog # K309-100; 100 isolations; Store at Multiple Temperatures)

I. Introduction:

Bacteria are one of the most abundant and diverse organisms on the planet, which take part in numerous critical ecosystem processes. Many bacterial species are pathogens that are responsible for causing a variety of human and animal diseases. In addition to their medical and ecological importance, bacteria are also used in various industrial applications such as production of enzymes and biofuels. BioVision's bacterial genomic DNA isolation kit provides convenient and simple step-by-step method for isolating quality genomic DNA from gram-negative and gram-positive bacterial species. This kit utilizes enzymatic reactions to release bacterial DNA from the cell. DNA release from the cell is coupled with adsorption of DNA onto a silica spin-column in the presence of high salt concentration, eliminating the use of toxic organic compounds or solvents. DNA purified by this kit is suitable for various downstream molecular biology applications such as PCR, cloning, DNA hybridization, and Southern Blotting.

II. Applications:

- PCR
- Cloning
- DNA Hybridization
- Southern Blotting

III. Sample Type:

- Gram positive and gram negative bacterial species

IV. Kit Contents:

Components	K309-100	Cap Code	Part Number	Storage (°C)
Buffer A [Re-suspension Buffer]	25 ml	NM/Clear	K309-100-1	RT
Enzyme Mix A	1 ml	Green	K309-100-2	-20°C
RNAse A	600 µl	Blue	K309-100-3	-20°C
Buffer B [Reaction Buffer]	1.7 ml	Yellow	K309-100-4	RT
Enzyme Mix B	1.2 ml	Red	K309-100-5	-20°C
Buffer C [Binding Buffer]*	25 ml	NM/Brown	K309-100-6	RT
Buffer D [Wash Buffer]**	30 ml	WM	K309-100-7	RT
Buffer E [Elution Buffer]	22 ml	WM	K309-100-8	RT
Spin Columns/Collection Tubes	100 tubes	-	K309-100-9	RT

Add 28 mL 100% Ethanol to the Buffer C [Binding Buffer] bottle and **136 mL 100% Ethanol to the Buffer D [Wash Buffer] bottle before use.

V. User Supplied Reagents and Equipment:

- DNAse-free aerosol tips and micro-centrifuge tubes
- 100% Ethanol
- Heating Block
- Centrifuge
- Mutanolysin and Lysostaphin

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and room temperature (RT), protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Buffer A, B,** and **E:** Ready to use. Store at RT.
- **Buffer C:** Add 28 ml of 100% Ethanol, molecular biology grade. Mix well and store at RT.
- **Buffer D:** Add 136 ml of 100% Ethanol, molecular biology grade. Mix well and store at RT.
- **Enzyme Mix A, Enzyme Mix B,** and **RNAseA:** Ready to use. Store at -20°C. Keep on ice at all times while in use.
- **Spin Columns:** Ready to use. Store at RT in dry conditions.

VII. Bacterial Genomic DNA Extraction Protocol:

1. Sample Preparation:

a. Transfer 1ml of overnight bacterial culture into a 1.5 ml tube and pellet the cells by centrifugation at 12,000 x g for 1 min at 4°C. Discard supernatant.

Note: If using >1-1.5 ml overnight culture, scale up the entire protocol proportionally.

b. Re-suspend the pellet in 250 µl Buffer A [Re-suspension Buffer].

A B C D

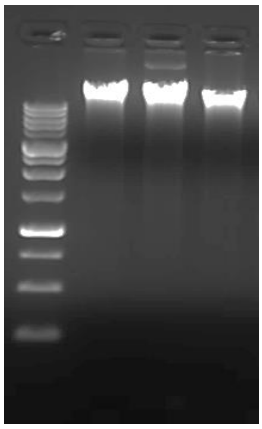


Figure 1.

1% 1X TBE agarose gel

Figure 1. Example genomic DNA isolation using BioVision's Bacterial Genomic DNA Isolation Kit. BioVision's Bacterial Genomic DNA Isolation kit efficiently purifies high quality genomic DNA from gram negative bacteria (such as *Escherichia* coli) and gram positive bacteria (such as *Staphylococcus* aureus and *Micrococcus* luteus). A: BriteRuler 1kb DNA Ladder; B: *M. luteus;* C: *S. aureus;* D*: E. coli.*

c. Add 10 µl of Enzyme Mix A. If RNA free DNA is desired, add 6 µl of RNAse A solution at this time. Mix by inverting the tube 3-5 times.

Note: Certain bacterial species require additional enzymes during cell lysis. For *Streptococcus mutans* and *Staphylococcus species*, supplement the lysis reaction with ≥ 250 units/mL Mutanolysin* and ≥ 200 units/mL Lysostaphin* respectively. *Not supplied.

d. Incubate for 30 min at 37°C.

2. DNA Release:

e. Add 17 µl of Buffer B [Reaction Buffer] and 12 µl of Enzyme Mix B. Mix by inverting the tube 3-5 times.

f. Incubate for 30 min at 55°C.

3. Binding:

g. Add 500 µl of Buffer C [Binding Buffer] and mix by pipetting up and down until the solution becomes clear.

h. Place the provided spin column into the provided collection tube and pipette the entire supernatant onto the top of the column.

i. Spin at 12,000 x g for 1 min at 4°C and discard the flow through.

4. Washing:

j. Add 750 µl of Buffer D [Wash Buffer] onto the top of the spin column and spin (12,000 x g; 1 min., 4°C). Discard the flow through.

k. Repeat step j one more time.

l. Spin the spin column at 12,000 x g for 2 min at 4°C to dry.

5. Elution:

m. Transfer the spin column to a clean, DNAse-free 1.5ml tube.

n. Add 200 µl of Buffer E [Elution Buffer] to the top of the spin column and incubate for 1-2 min at RT.

o. Spin at 12,000 x g for 1 min at 4°C to elute the genomic DNA.

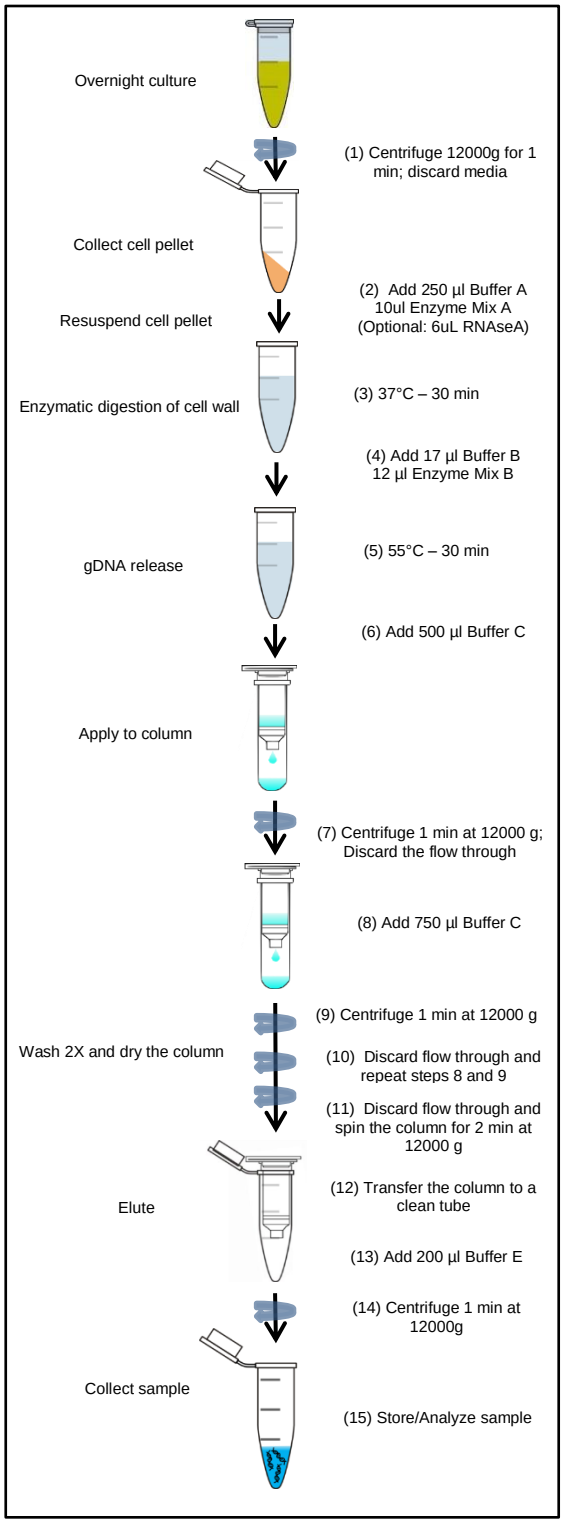
p. Sample is now purified and ready to use. Store genomic DNA at -20°C or immediately use the sample in a downstream application of your choice.

Note: *(Note: Generally, good quality genomic DNA will have A260/280 of 1.7- 1.99 and exhibit one clear band of high molecular weight on 1% agarose gel. See troubleshooting guide in Section VIII for help

VIII. Troubleshooting:

Issue	Possible Reason	Recommendations
Low yield	Low bacteria concentration	Monitor $OD_{600\ nm}$ of your overnight culture. We recommend $OD_{600\ nm}$ range to be between 1 and 2.
	Incomplete Lysis	Increase incubation with Enzyme Mix A up to 45 min – 1hr.
	Incomplete DNA release	Increase incubation with Enzyme Mix B up to 45 min - 1hr.
Low A260/280 (<1.6)	Protein Contamination	Increase incubation with Enzyme Mix B up to 45 min - 1hr.
High A260/280 (>2.0)	RNA Contamination	Add RNAse A during the cell lysis step.
No DNA band/severe smear on gel	DNAse contamination	Use DNAse free aerosol tips, DNAse-free tubes, and practice good sterile technique

Protocol Quick Guide



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